

Attorney Docket No.: **PM (DC-0251)**
Inventor: **Wade and Demain**
Serial No.: **09/720,078**
Filing Date: **July 25, 2001**
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Amendments to the Specification:

Please replace the paragraph bridging pages 2 and 3 with the following rewritten paragraph:

--While the explanation for the reduced efficacy of the aged immune system is unresolved, it appears to involve the impairment of different immune functions, including various T-cell functions. Based thereon, T-cells from aged individuals have been extensively studied in an effort to explain and obviate impairments in T-cell activation associated with aging. The results of these studies have indicated that T-cell proliferation, generation of CTL effectors, and the delivery of T-cell helpers for B-cell induction is reduced in aged individuals. (Miller, R.A. (Id.).) It has been hypothesized that the reduced efficacy of T-cells in aged individuals to elicit an effective immune response may be attributable to the reduced production of IL-2. (Thomas Thoman et al, *J. Immunol.*, 127:2101-2106 (1981).) --

Please replace the paragraph bridging pages 10 and 11 with the following rewritten paragraph:

--The antigen which is directly or indirectly fused to the antibody will typically comprise an antigen which is specific to an etiological agent, for example, an antigen expressed by or a product of a tumor cell, a virus, a bacterium, a parasite, or other infectious agent. Examples thereof, for example, purified recombinant proteins, peptides, whole organisms such as intact

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~~virons~~ virions, flu, polio, tetanus and diphtheria toxins, TB antigens, and HIV gp120 antigens, as well as tumor antigens such as breast, ovarian and prostate tumor antigens. Specific examples thereof include, by way of example, antigens expressed by HIV such as gp160, Gag, Pol, New, The, and Reb; malarial antigens such as the CS protein and ~~sporozoite~~ sporozoite surface protein 2; hepatitis B surface antigens such as pre-S1, pre-S2, HBc Ag, and Hbe Ag; influenza antigens such as HA, NP and NA; hepatitis A surface antigens; hepatitis C surface antigens; herpes virus antigens, such as EBV GP340, EBV GP85, HSV gB, GSV gD, HSV gH, HSV early protein product, cytomegalovirus gB, cytomegalovirus gH, and IE protein gp72, ~~respiratorial~~ and gp72; respiratory syncytial viral antigens such as the F protein, G protein and N protein; leprosy antigens; listeriosis antigens; tumor antigens such as carcinoma CA, carcinoma mutated EGF receptor, prostate carcinoma specific antigen (PSA), prostate specific membrane associated antigen, carcinoma associated mucin, carcinoma p21, carcinoma p53, melanoma MPG, melanoma p97, MAGE-1, MAGE 3, gp100, MART 1, melanoma antigen gp75, carcinoma NEU oncogene product, and ras protein. Other examples include papillomavirus antigens such as the L1 and L2 proteins, and Lyme's disease antigens. Typically the antigen will be one expressed on the surface of a particular target, for example, a tumor cell, a pathogen, a bacterium or a virally infected cell.--

Please replace the following paragraphs beginning on page 18, line 3 with the following rewritten paragraphs:

--Hen egg lysozyme/anti-class II conjugates were produced as described by Snider et al. (Snider et al (Id.)). Briefly, HEL

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(Sigma Chemical Co., St. Louis, MO) and protein A, purified anti I-A^k mAb (10.2.16) were substituted with the heterobifunctional cross-linking reagent N-succinimidyl 3-(2-pyridyldithio)propionate (Pierce Chemicals, Rockford, IL) and reacted to form heteroconjugates. The HEL/anti-class II complexes were separated from free HEL by ~~Sephadex~~ SEPHADEX® G75 chromatography to yield a class II targeting construct with a final antigen concentration of 320 µg/mL. Avidin/mAb conjugates were produced by biotinyllating 500 µg of purified mAbs: anti-class II (10.2.16), anti-CD38, anti-CD40 (FGK 115) or anti-CD11c with 55 µg biotin (Pierce Chemicals, Rockford, IL). The 10.2.16 (Ui Oi et al, Curr. Topics Microbiol Immunol., and 81:115-129 (1990)), FGK-115 mAb (a gift from Dr. G. Roinik, Basal, Switzerland) were produced in-house by protein-A affinity chromatography. Biotinylated mAbs were dialyzed against PBS (pH 7.4) overnight at 4°C before conjugation with 652.6 µg avidin (Sigma Chemical Co., St. Louis, MO). Antigen/mAb conjugates and titrated amounts of free HEL or avidin were separated on 14% SDS-PAGE gels under reducing and non-reducing conditions to determine the amount of bound and unbound antigen in conjugate preparation.

Immunizing Reagents

Immunizing inoculum for all experiments was prepared in PBS (pH 7.4) in a final volume of 100 µL and then sterile filtered (0.2 µm syringe filters, ~~Millipore~~ MILLIPORE®, Bedford, MA) before subcutaneous or intraperitoneal inoculations. For *in vitro* antigen loading of DC, a spleen was removed from either an aged or young mouse that was treated for 10 days with human recombinant Flt-3 Ligand [Pulendran et al, E. Developmental pathways of dendritic cells *in vivo*: distinct function, phenotype

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and localization of dendritic cell subsets in FLT3 ligand-treated mice, *J. Immunol.*, 159:2222-2231 (1997); Immunex Corp., Seattle, WA.] Single cell suspensions were made by passage through sterile nylon dialysis mesh; red blood cells were lysed with ammonium chloride and cell suspensions were T-cell-depleted using a cocktail of anti-CD4 mAb, anti-Thy1.1 mAB plus rabbit complement (~~Low-Tox~~ LOW-TOX®; Accurate Chemical Co., Westbury, NY).

B-cell depletion was effected by anti-IgM panning for 60 minutes at 37°C (~~Zymed~~ ZYMED® Laboratories, Inc., So San Francisco, CA). (Macrophages stick to the panning plates, however, Flt-3 treated DC do not (W. F. Wade, personal observation).) In our hands, the purity of DC obtained from young and old mice by this method was about 45%, as evidenced by CD11c staining (data not shown). HEL/anti-class II conjugate was added to cells resuspended in ~300 µl volume, incubated at 4°C for 30 minutes and washed twice with PBS (pH 7.4). Fifty million cells were plated at 10⁶/mL, incubated at 37°C in 5% CO₂ for four hours prior to the addition of 10 µg/ml anti-CD40 mAb or 3.2 µg soluble HEL and incubated overnight. Cells were ~~scrapped~~ scraped, washed one time in PBS, counted and inoculated into age-matched mice subcutaneously along the right flank.

Animal Manipulation

Young (8-12 weeks) and old (15-19 months) female CBA/jNIA mice were purchased from the National Institute of Aging mouse colony. They were housed in the Animal Resources Center at Dartmouth Medical School where they were provided with TekLad rodent diet (Harlan, Madison, WI) and water *ad libitum*. Mice were anesthetized using ~~Methophane~~ METOPHANE™ (Mallinckrodt Veterinary, Inc., Mundelein, IL) and inoculated with sterile

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solutions using tuberculin syringes fitted with a 27 ga. Needle on the right flank for subcutaneous inoculations or the right side of ventral centerline for intraperitoneal inoculations. Blood collection at different time points in the experiments were performed on anesthetized mice through the retro-orbital sinus. Immunizing inoculations were performed on day 0 followed by serum collection to assess primary responses at +21 days. Mice were challenged subcutaneously with either 25 µg of soluble HEL or avidin 14 days later (+35d) and bled for secondary response antisera on +45 days. In one experiment, the mice were challenged intravenously with 25 µg of soluble avidin.--